UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/553,307	08/07/2006	John C. Gebler	64254(49991)	1965

48990 7590 06/09/2010

EDWARDS ANGELL PALMER & DODGE LLP
P.O. BOX 55874
BOSTON, MA 02205

EXAMINER	
XU, XIAOYUN	

ART UNIT	PAPER NUMBER
1797	

MAIL DATE	DELIVERY MODE
06/09/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/553,307	GEBLER ET AL.	
	Examiner	Art Unit	
	ROBERT XU	1797	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 May 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *See Continuation Sheet* is/are pending in the application.

4a) Of the above claim(s) *See Continuation Sheet* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 2, 51, 56, 57, 65, 73, 77, 79, 83, 88, and 99-102* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continuation of Disposition of Claims: Claims pending in the application are 1,2,4-13,15-18,48, 49, 51,52,56-60,65,66,70-73,77,79,83-88,90,91,93 and 99-102.
Continuation of Disposition of Claims: Claims withdrawn from consideration are 4-13,15-18,48,49,52,58-60,66,70-72,84-87,90,91 and 93.

DETAILED ACTION

1. The amendment and RCE filed on 05/14/2010 has been entered and fully considered. Claims 23, 24, 37, 41, 44 and 50 are canceled. Claims 1, 2, 4-13,15-18, 48, 49, 51, 52, 56-60, 65, 66, 70-73, 77, 79, 83-88, 90, 91, 93 and 99-102 are pending, of which claims 1 and 65 are amended, claims 4-13,15-18,48,49,52,58-60,66,70-72,84-87,90,91 and 93 are withdrawn, claims 1, 2, 51, 56, 57, 65, 73, 77, 79, 83, 88, and 99-102 are considered over merits.

Response to Amendment

2. In response to amendment, the examiner withdraws objection and modifies rejection over the prior art established in the previous Office action.

Claim Rejections - 35 USC § 103

3. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

4. **Claims 1, 2, 37, 51, 56, 57, 65, 73, 77, 79, 83, 88 and 99** are rejected under 35 U.S.C. 103(a) as being unpatentable over Huang et al (Analytical Biochemistry, 1999, IDS) (Huang) and as evidenced by Imaizumi et al. (Journal of Physical Chemistry, 1995) (Imaizumi) and Xu et al. (Tetrahedron Letters, 2001) (Xu).

In regard to Claim 1, Huang teaches a method of preparing a sample for mass spectrometry analysis. The method comprises:

a) obtaining a sample comprising an analyte (peptide from tryptic digested protein), the analyte comprises an exposed group (NH_2-terminus) (see page 307, right col. 2nd paragraph); and

b) reacting the analyte (peptide) with a triarylphosphonium labeling reagent (Tris(trimethoxyphenyl)phosphonium (TMPP) reagents) having a reactive group (acetyl-O-succinimide (AcOSu)) capable of reacting with the exposed group (NH_2-terminus) to form a triarylphosphonium-linked analyte (see page 307, right col. 3rd paragraph).

wherein the labeling reagent has a structure according to the formula

$[Ar_3P^+R]X^-$,

wherein

each Ar is an aryl group (methoxyphenyl) (see Scheme 1);

P is a phosphorous atom (see Scheme 1);

R is a reactive group comprising a functional group (AcOSu) that react with the exposed group (NH2-terminus) to form a covalent bond thereby forming triarylphosphonium-linked analytes (TMPP-Ac-peptide) (see Scheme 1); and

X^- is a negatively-charged counter ion (Br^-) (see Scheme 1).

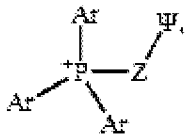
Huang does not teach that the Ar_3P group is selected from the group consisting of unsubstituted naphthyldiphenylphosphine, dinaphthylphenylphosphine, trinaphthylphosphine, 9- anthryldiphenylphosphine, 9-anthryldinaphthylphosphine, diphenylpyrenylphosphine, dinaphthylpyrenylphosphine. Huang teaches that Ar_3P is Tris(trimethoxyphenyl)phospine. The positive charge of Tris(trimethoxyphenyl)phospine is stabilized by the electron-donation from methoxyl substitute through the phenyl. Naphthyl, anthryl and pyrenyl have fused 2, 3, and 4 phenyl rings. They are known as fluorophores and have large conjugate orbital that can share electrons as evidenced by Imaizumi (see page 3810) and Xu (see page 9250). The conjugated system results in a general delocalization of the electrons across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the molecule. At time of the invention it would have been obvious to one of ordinary skill in the art to substitute trimethoxyphenyl with naphthyl, anthryl or pyrenyl in Huang's method in order to stabilize the phosphine by delocalizing the positive charge of phosphine. One would be motivated to do so because conjugated system of naphthyl, anthryl or pyrenyl would result in a general delocalization of the positive charge of phosphine across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the phosphine. The effect of the positive charge delocalization is similar to the effect of electron-donation. Trimethoxyphenyl (MW 168) is just as bulky as naphthyl (MW 128). Thus trimethoxyphenyl (MW 168) and naphthyl (MW 128) would be expected to have similar effect of a hindrance in the reactivity of these reagents with the analyte.

In regard to Claim 2, Huang teaches that the method comprising the further step of obtaining the triarylphosphonium labeling reagent having a reactive group (see page 307, left col. 3rd paragraph).

In regard to Claim 51, Huang teaches that the exposed group of the analyte (N-terminal group of peptide) is electrophilic and the reactive functional group (O-succinimide (OSu)) is nucleophilic (see scheme 1).

In regard to Claim 56, Huang teaches that X^- is a halide (Br^-) (see scheme 1).

In regard to Claim 65, Huang teaches that the labeling reagent has the following structure:



wherein

each Ar is aryl group (methoxyphenyl) (see scheme 1);

P is a phosphorous atom (see scheme 1);

Z is a linking group (Ac) (see scheme 1); and

Ψ is a reactive functional group (OSu) (see scheme 1).

As has been discussed in regard to Claim 1 above, Huang does not teach that the Ar_3P group is selected from the group consisting of unsubstituted naphthyldiphenylphosphine, dinaphthylphenylphosphine, trinaphthylphosphine, 9-anthryldiphenylphosphine, 9-anthryldinaphthylphosphine, diphenylpyrenylphosphine, dinaphthylpyrenylphosphine. Huang teaches that the Ar_3P is Tris(trimethoxyphenyl)phospine. The positive charge of Tris(trimethoxyphenyl)phospine is stabilized by the electron donated from trimethoxyl substitute through the phenyl. Naphthyl, anthryl and pyrenyl have fused 2, 3, and 4 phenyl rings. They are known as fluorophores and have large conjugate orbital that can share electrons as evidenced by Imaizumi and Xu. The conjugated system results in a general delocalization of the electrons across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the molecule. At time of the

invention it would have been obvious to one of ordinary skill in the art to substitute trimethoxyphenyl with naphthyl, anthryl or pyrenyl in Huang's method in order to stabilize the phosphine by delocalizing the positive charge of phosphine. One would be motivated to do so because conjugated system of naphthyl, anthryl or pyrenyl would result in a general delocalization of the positive charge of phosphine across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the phosphine. The effect of the positive charge delocalization is similar to the effect of electron-donation. Trimethoxyphenyl (MW 168) is just as bulky as naphthyl (MW 128). Thus trimethoxyphenyl (MW 168) and naphthyl (MW 128) would be expected to have similar effect of a hindrance in the reactivity of these reagents with the analyte.

In regard to Claim 73, Huang teaches that Ψ group is an isocynate (OSu) (see scheme 1).

In regard to Claim 77, Huang teaches that Ψ group is an aryl halide (SC_6F_5) (see scheme 1).

In regard to Claim 79, Huang teaches that Z has 3 nonhydrogen atoms selected from group consisting of C, N, O and S, and the longest liner segment contains 2 nonhydrogen atoms (see scheme 1).

In regard to Claim 83, Huang teaches that the analyte is a peptide (see scheme 1, page 307).

In regard to Claims 88 and 99, Huang does not specifically teach that the sample is a biological tissue. It is well known that proteins can be obtained from biological tissue. At the time of the invention it would have been obvious to one of ordinary skill in the art to analyzing a biological tissue that contains proteins.

6. **Claims 1, 88, 99 and 100** are rejected under 35 U.S.C. 103(a) as being unpatentable over Leavens et al (Rapid Communications in Mass Spectrometry, 2002, IDS) (Leavens) and as evidenced by Imaizumi and Xu.

In regard to Claim 1, Leavens teaches a method of preparing a sample for mass spectrometry analysis. The method comprises:

a) obtaining a sample comprising an analyte (amines) (see Table 1), the analyte comprises an exposed group (amine group) (see page 439, right col. 1st paragraph); and

b) reacting the analyte (amines) with a triarylphosphonium labeling reagent (TMPP-reagents) having a reactive group (carboxylic group) capable of reacting with the exposed group (amine group) to form a triarylphosphonium-linked analyte (see Table 3, page 439, right col. 1st paragraph).

wherein the labeling reagent has a structure according to the formula

$[Ar_3P^+R]X^-$,

wherein

each Ar is an aryl group (methoxyphenyl) (see Scheme 1);

P is a phosphorous atom (see Scheme 1);

R is a reactive group comprising a functional group (carboxylic group) that react with the exposed group (NH2-terminus) to form a covalent bond thereby forming triarylphosphonium-linked analytes (TMPP-Ac-peptide) (see Scheme 1); and

X^- is a negatively-charged counter ion (Br^-) (see Scheme 1).

Leavens does not teach that the Ar_3P group is selected from the group consisting of unsubstituted naphthyldiphenylphosphine, dinaphthylphenylphosphine, trinaphthylphosphine, 9- anthryldiphenylphosphine, 9-anthryldinaphthylphosphine, diphenylpyrenylphosphine, dinaphthylpyrenylphosphine. Leavens teaches that the Ar_3P is Tris(trimethoxyphenyl)phospine. The positive charge of Tris(trimethoxyphenyl)phospine is stabilized by the electron donated from trimethoxyl substitute to the phenyl. Naphthyl, anthryl and pyrenyl have fused 2, 3, and 4 phenyl rings. They are known as fluorophores and have large conjugate orbital that can share electrons as evidenced by Imaizumi (see page 3810) and Xu (see page 9250). The conjugated system results in a general delocalization of the electrons across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the molecule. At time of the invention it would have been obvious to one of ordinary skill in the art to substitute trimethoxyphenyl with naphthyl, anthryl or pyrenyl in Leavens' method in order to stabilize the phosphine by delocalizing

the positive charge of phosphine. One would be motivated to do so because conjugated system of naphthyl, anthryl or pyrenyl would result in a general delocalization of the positive charge of phosphine across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the phosphine. The effect of the positive charge delocalization is similar to the effect of electron-donation. Trimethoxyphenyl (MW 168) is just as bulky as naphthyl (MW 128). Thus trimethoxyphenyl (MW 168) and naphthyl (MW 128) would be expected to have similar effect of a hindrance in the reactivity of these reagents with the analyte.

In regard to Claims 88 and 99, Leavens teaches a method of preparing a sample for mass spectrometry analysis, comprising

a) obtaining a sample comprising an analyte (amines) having an exposed group (amine group) (see page 439, right col. 1st paragraph, Table 1); and

b) reacting the analyte (amines) with a triarylphosphonium labeling reagent (TMPP-reagents) having a reactive group (carboxylic group) capable of reacting with the exposed group (amine group) to form a triarylphosphonium-linked analyte (see Table 3, page 439, right col. 1st paragraph).

Leavens does not specifically teach that the sample is a biological tissue. It is well known that biological tissue contains amines as well as other molecules. At the time of the invention it would have been obvious to one of ordinary skill in the art to take the advantage of the labeling agent in analyzing a biological tissue that contains amines. One would be motivated to do so because the labeling reagent can specifically react with amines in a biological tissue and makes the specific detection of amines in a biological tissue much easier.

In regard to Claim 100, Leavens teaches that the analyte is a small molecule (amines) (see Table 1).

Response to Arguments

7. Applicant's arguments filed 05/14/2010 have been fully considered but they are not persuasive.

Applicant submits that one of ordinary skill in the art would recognize that the electron-donation of the methoxy groups would likely make the TMPP reagents stronger Lewis bases than the unsubstituted, bulky groups of the compounds of the instant claims. That is, their ability to donate electrons and thereby associate with the analyte would be expected to be stronger. Furthermore, the steric bulk of the compounds of the instant claims would be expected to be a hindrance in the reactivity of these reagents with the analyte.

First, trimethoxyphenyl (MW 168) is just as bulky as naphthyl (MW 128). Thus trimethoxyphenyl (MW 168) and naphthyl (MW 128) would be expected to have similar effect of a hindrance in the reactivity of these reagents with the analyte.

Second, Naphthyl, anthryl and pyrenyl have fused 2, 3, and 4 phenyl rings. They are known as fluorophores and have large conjugate orbital that can share electrons as evidenced by Imaizumi and Xu. The conjugated system results in a general delocalization of the electrons across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the molecule. At time of the invention it would have been obvious to one of ordinary skill in the art to substitute trimethoxyphenyl with naphthyl, anthryl or pyrenyl in Leavens' method in order to stabilize the phosphine by delocalizing the positive charge of phosphine. One would be motivated to do so because conjugated system of naphthyl, anthryl or pyrenyl would result in a general delocalization of the positive charge of phosphine across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the phosphine. The effect of the positive charge delocalization is similar to the effect of electron-donation.

Applicant argues that Huang or Leavens does not teach analyzing biological tissue sample. Huang teaches analyzing peptide that has an exposed functional group that reacts with the reactive group of the labeling reagent. Leavens teaches analyzing amines and carboxylic acid compounds that react with the reactive group of the labeling reagent (see Table 1 & 2). Many biological samples contain peptides, amines or carboxylic acids as well as other molecules. It would have been obvious for a routineer to take the advantage of the labeling reagent in analyzing a biological tissue sample that

contains peptides or amines or carboxylic acids or any molecules that contains functional group that reacts with the reactive group of the labeling reagent as taught by Huang or Leavens. One would be motivated to do so because the labeling reagent can specifically react with amines in a biological tissue and makes the specific detection of amines in a biological tissue much easier.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ROBERT XU whose telephone number is (571)270-5560. The examiner can normally be reached on Mon-Thur 7:30am-5:00pm, Fri 7:30am-4:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Vickie Kim can be reached on (571)272-0579. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

6/4/2010

/Yelena G. Gakh/
Primary Examiner, Art Unit 1797

RX